Combined effects of smoking, anti-EBNA antibodies, and *HLA-DRB1*1501* on multiple sclerosis risk

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ABSTRACT

Objective: To examine the interplay between smoking, serum antibody titers to the Epstein-Barr virus nuclear antigens (anti-EBNA), and *HLA-DR15* on multiple sclerosis (MS) risk.

Methods: Individual and pooled analyses were conducted among 442 cases and 865 controls from 3 MS case-control studies—a nested case-control study in the Nurses' Health Study/Nurses' Health Study II, the Tasmanian MS Study, and a Swedish MS Study. Conditional logistic regression models were used to calculate odds ratios (ORs) and 95% CIs for the association between smoking, anti-EBNA titers, *HLA-DR15,* and MS risk. Study estimates were pooled using inverse variance weights to determine a combined effect and *p* value.

Results: Among MS cases, anti-EBNA titers were significantly higher in ever smokers compared to never smokers. The increased risk of MS associated with high anti-EBNA Ab titers was stronger among ever smokers (OR = 3.9, 95% CI = 2.7–5.7) compared to never smokers (OR = 1.8, 95% CI = 1.4–2.3; *p* for interaction = 0.001). The increased risk of MS associated with a history of smoking was no longer evident after adjustment for anti-EBNA Ab titers. No modification or confounding by *HLA-DR15* was observed. The increased risk of MS associated with ever smoking was only observed among those who had high anti-EBNA titers (OR = 1.7, 95% CI = 1.1–2.6).

Conclusions: Smoking appears to enhance the association between high anti-EBNA titer and increased multiple sclerosis (MS) risk. The association between *HLA-DR15* and MS risk is independent of smoking. Further work is necessary to elucidate possible biologic mechanisms to explain this finding. ***Neurology*® 2010;74:1365–1371**

GLOSSARY

anti-EBNA = antibody titers to the Epstein-Barr virus nuclear antigens; **EBV** = Epstein-Barr virus; **MS** = multiple sclerosis; **NHS** = Nurses' Health Study; **NHSII** = Nurses' Health Study II; **NSHDS** = Northern Sweden Health and Disease Study Cohort; **OR** = odds ratio.

Multiple sclerosis (MS) is the most common nontraumatic disabling neurologic disease among young adults in the United States.[1] The exact mechanism resulting in disease progression is unknown, though likely autoimmune in origin. Risk factors that have been consistently associated with increased MS risk include the *HLA-DRB1*1501* haplotype in Caucasian populations,[2] high antibody levels against the Epstein-Barr virus (EBV),[3] and a smoking history.[4] Whether these factors are independently associated with risk of MS or are related, possibly suggesting a common biologic mechanism, is unclear. We recently observed that the associations between *HLA-DR15* positivity and EBV infection are independent MS risk factors.[5,6] However, the risk of MS associated with smoking independent of the other 2 factors and potential 3-way relationships have not been investigated.

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To address these questions, we combined data from the Nurses' Health Studies, the Tasmanian MS Study, and a Swedish MS Study to assess the association of these risk factors with MS, simultaneously considering the interplay between them, to further elucidate plausible biologic mechanisms.

METHODS

Study population. This study included participants in 3 studies: the Nurses' Health Study (NHS)/Nurses' Health Study II (NHSII), the Tasmanian MS Study, and the Swedish MS Study.

Nurses' Health Studies. The study population includes women who returned blood or buccal swab samples among participants in 2 ongoing, prospective cohorts: the NHS and NHSII. Information on lifestyle factors and disease history is obtained via biennial questionnaire. The details of MS case documentation have been previously described.[7] Among women with biologic samples, we documented 217 incident MS cases (149 with blood and 68 with buccal cell swabs) and matched 2 controls by age and study. Because DNA samples were collected during the general cohort follow-up, some cases had blood collected before symptom onset (n = 18), with the majority collected after symptom onset (n = 128). All were incident with respect to collection of smoking information.

Tasmanian MS Study. This is a population-based study using prevalent cases.[8,9] Cases were identified through a variety of population-based strategies, including information sessions at MS societies and invitation letters from neurologists to patients. Two community controls were selected from a voter registration for compulsory political elections and matched to each case by sex and birth year (response rate = 76%). The study population included 136 cases and 272 controls.

Swedish MS Study. Cases and controls were previously selected for a study on MS risk and EBV Ab titers.[10] Individuals with MS were identified from a national registry and, concurrently, blood specimens were collected as part of a population-based study, the Northern Sweden Health and Disease Study Cohort (NSHDS). Individuals from the MS registry were linked to the NSHDS and 2 controls matched by gender, age, and year of blood collection, resulting in 110 cases and 220 controls. Because biologic samples were collected at a variety of time points, some MS cases are retrospective (onset prior to blood draw, n = 84) and others prospective (no evidence of clinical symptoms prior to blood draw, n = 12).

In total, the 3 studies included 463 cases and 920 controls. The final pooled study population included 442 cases and 865 controls with information on smoking history (NHS/NHSII: 210 cases, 420 controls; Tasmanian: 136 cases, 272 controls; Swedish: 96 cases, 173 controls). Numbers in specific analyses may not sum to the total number of cases due to individuals missing relevant information and these deviations are noted in appropriate tables or text.

Standard protocol approvals, registrations, and patient consents. This study was approved by local or institutional ethical standards committees as appropriate.

Assessment of smoking. ***Nurses' Health Studies.*** Information on smoking status (current, past, never) and quantity (cigarettes per day) were collected via biennial questionnaire. To categorize smoking at blood draw, smoking information closest in time to and preceding the blood draw was used. Similarly, for smoking at time of diagnosis, smoking status nearest in time and preceding the diagnosis date was used.

Tasmanian MS Study. Smoking history was assessed through structured nurse-administered interview. Smoking information obtained at the blood draw date (interview date) included current smoker (yes/no). Further information on number of cigarettes per day and duration of use was collected. Participants were asked whether they were ever or never smokers at the time of MS diagnosis. Smoking status at blood draw was assigned as current/past/never, as follows: assuming that no participants reporting never smoking at diagnosis subsequently began smoking and then quit, nonsmokers at blood draw who were never smokers were assumed to be never smokers at blood draw. Individuals who reported ever smoking at diagnosis and nonsmoking at blood draw were classified as past smokers and those reporting current smoking at blood draw were considered current smokers.

Swedish MS Study. Smoking history was assessed via self-administered questionnaire at time of enrollment in the NSHDS.[11] Participants were asked if they currently smoked and, if not, if they had ever smoked. Smoking is only available for the time of blood draw. To address the potential impact on results, in the NHS, subanalyses were performed comparing the use of smoking at blood draw and smoking at date of diagnosis and results were nearly identical.

Assessment of anti-EBNA titers. The measurement of anti-EBNA1 immunoglobulin G antibodies titers in the NHS/NHSII MS cases and controls was conducted for a previous study[12] using an anticomplement immunofluorescence assay.[13] In the Swedish study, anti-EBNA1 IgG antibody titers were measured using an ELISA assay (Biotest, Germany).[10] In the Tasmanian study, total anti-EBNA IgG titers were measured using an ELISA assay (Panbio, Brisbane, Australia).[9]

Notably, 2 studies measured anti-EBNA-1, while the third measured total anti-EBNA. To address the appropriateness of combining these data, we examined the relationship in the NHS cohorts, where information is available on both EBNA-1 and total EBNA. In the NHS/NHSII, the correlation between EBNA-1 and EBNA is very high ($r = 0.87$). Further, main analyses were repeated in the NHS/NHSII comparing results using total EBNA vs EBNA-1 and we obtained nearly identical estimates.

Assessment of HLA. In the NHS/NHSII and Tasmanian studies, a surrogate SNP (rs3135005), which is highly correlated with *HLA DRB1*1501*, was used as a marker for *HLA-DR15* positivity.[5] In the Swedish study, a low-resolution DR kit[6] was used for typing and, therefore, individuals were classified as *HLA-DR15* positive or negative.

Statistical analyses. ***Smoking classification.*** For descriptive characteristics and associations separately among cases and controls (tables 1 and 2), smoking at blood draw was classified as current/past/never. For main analyses, participants were classified as ever/never smokers based on date of diagnosis for the NHS/NHSII and Tasmanian studies, and date of blood draw in the Swedish study.

EBNA titer classification. Anti-EBNA Ab titers were standardized to a normal distribution (mean = 0, SD = 1) using the mean and SD among the controls within each cohort. For stratification by anti-EBNA titer, within each cohort, Ab titers were divided into quartiles or dichotomized at the median of the distribution among controls.

HLA-DR15 classification. *HLA-DR15* was considered as a dichotomous variable (positive/negative).

Statistical models. *EBNA titers,* HLA-DR15, *and smoking among cases and controls.* Generalized linear models adjusting for

Table 1 Characteristics of participants at time of blood draw according to study[a,b]

	Nurses' Health Studies		Tasmanian MS Study		Swedish MS Study	
	Cases	Controls	Cases	Controls	Cases	Controls
No. of subjects	210	420	136	272	96	173
Mean age at blood collection, y	53.3	53.4	43.9	43.9	48.0	47.7
Women, n (%)	210 (100)	420 (100)	92 (68)	184 (68)	55 (57)	95 (55)
HLA-DR15 positive, n (%)	113 (55)	126 (30)	77 (57)	66 (28)	63 (66)	59 (34)
Smoking status, n (%)						
Current	21 (10)	42 (10)	43 (32)	64 (24)	31 (32)	52 (30)
Past	104 (50)	175 (42)	43 (32)	79 (29)	22 (23)	27 (16)
Never	85 (40)	203 (48)	49 (36)	129 (47)	43 (45)	94 (54)
Anti-EBNA (standardized mean ± SD)[c]	0.45 ± 0.72[d]	0 ± 1[d]	0.78 ± 0.57	0 ± 1	0.58 ± 0.53	0 ± 1

Abbreviations: EBNA = Epstein-Barr virus nuclear antigens; MS = multiple sclerosis; NHS = Nurses' Health Study.
[a]Race/ancestry—Complete data are only available in the NHS/NHSII, where 5% report nonwhite ancestry (1% Hispanic). In the Swedish study, information is only available for cases where 99% report being Caucasian. Though specific ancestry data are not available, a requirement for inclusion in the study was at least one grandparent being born in Tasmania, and no participants had dark skin based on spectrophotometry, suggesting all participants were Caucasian.
[b]Among those with smoking information.
[c]EBNA1 for NHS/NHSII and Swedish MS Study; total EBNA for Tasmanian MS Study.
[d]Only among women with blood sample and serologic data available (n = 146 cases, 292 matched controls).

the matching factors (age and gender) were used to assess the association between anti-EBNA Ab titers or *HLA-DR15* and smoking history (current, past, never).

MS risk and EBNA titers, smoking history, and HLA-DR15 *positivity.* Consistent with the matched design, conditional logistic regression models were used to calculate odds ratios (ORs) and 95% CIs for the association among anti-EBNA titers (standardized), smoking history (ever/never), and *HLA-DR15* (positive/negative) and risk of MS. Two-way interactions were investigated by considering stratum-specific estimates and evaluating the heterogeneity using interaction terms which were the cross-product of the 2 relevant variables. Additionally, combined effects were calculated for factors for which a significant interaction was observed. For missing values of anti-EBNA titers or *HLA-DR15*, an indicator term was included in models (though women in the NHS with DNA from cheek swabs were excluded from analyses including anti-EBNA Ab titers as discussed in Results).

Study-specific estimates were pooled to determine a combined OR and 95% CI using inverse variance weights.[14] Fixed effect model estimates were used when tests of heterogeneity between studies were nonsignificant at the $\alpha = 0.05$ level; otherwise, random effects models were used as noted.

RESULTS

Population characteristics. Characteristics of MS cases and matched controls are shown in table 1. Notably, the Tasmanian and Swedish studies include men, while the NHS/NHSII includes only women. Although rates of current and past smoking varied, the proportion of ever smokers was similar among the 3 studies.

EBNA titers, HLA-DR15, and smoking. Comparing standardized anti-EBNA Ab titers among current, past, and never smokers, ever smoking in MS cases appeared to be consistently related to increased Ab titers in all 3 studies, though this was not significant in the Swedish study. Among controls, the association between a smoking history and higher anti-EBNA Ab titers was significant in the Tasmanian study, but not in the other studies. No association was observed between *HLA-DR15* and smoking status among cases or controls (table 2).

EBNA titers, HLA-DR15, smoking, and risk of MS. As previously reported, there was a univariate association between smoking, anti-EBNA Ab titer, or *HLA-DR15* positivity and MS, although some study-specific estimates were nonsignificant ($p \geq 0.05$) (table 3). A consistent 50% increased risk of MS associated with ever smoking was observed (table 3). To examine the effect of smoking, independent from that of anti-EBNA titers, we compared the association between ever smoking and MS risk with and without adjustment for anti-EBNA Ab titer (this excludes the women in the NHS cohorts who do not have a blood sample). Similar to the results presented in table 3 (which only includes NHS/NHSII participants who had DNA), there was a 40% increased MS risk associated with ever smoking (pooled OR = 1.4, 95% CI = 1.1–1.8; $p = 0.02$). However, upon adjustment for anti-EBNA Ab titer, the apparent association between ever smoking and MS was no longer evident (OR = 1.1, 95% CI = 0.8–1.4; $p = 0.52$). Results of secondary analyses using pack-years of smoking were similar—MS risk increased by 15% for each 10-year increment in pack-years ($p = 0.02$), but this association was attenuated after adjustment for anti-EBNA titers ($p = 0.49$).

Interaction between EBNA titers, HLA-DR15, and smoking. The increased risk of MS associated with increasing anti-EBNA Ab titers was twice as strong

Table 2 Mean anti-EBNA Ab titers and percent *HLA-DR15* positivity according to smoking status at time of blood draw in cases and controls

	Smoking status			
	Never	Past	Current	*p* Value
Anti-EBNA Ab titer[a] (standardized mean)				
Nurses' Health Studies				
Cases	0.24 ± 0.76	0.61 ± 0.66	0.65 ± 0.66	0.005
Controls	−0.06 ± 1.07	0.04 ± 0.90	0.03 ± 1.02	0.38
Tasmanian MS Study				
Cases	0.56 ± 0.64	0.92 ± 0.42	0.87 ± 0.56	0.01
Controls	−0.19 ± 1.04	0.05 ± 0.97	0.31 ± 0.88	0.007
Swedish MS Study				
Cases	0.49 ± 0.70	0.63 ± 0.33	0.67 ± 0.34	0.29
Controls	0.01 ± 1.08	−0.17 ± 1.07	0.08 ± 0.92	0.65
HLA-DR15 positivity (n)				
Nurses' Health Studies				
Cases	51% (43)	57% (57)	62% (13)	0.59
Controls	35% (70)	25% (43)	32% (13)	0.07
Tasmanian MS Study				
Cases	61% (30)	58% (25)	51% (22)	0.47
Controls	25% (28)	31% (22)	32% (16)	0.45
Swedish MS Study				
Cases	72% (31)	55% (12)	65% (20)	0.32
Controls	34% (32)	26% (7)	38% (20)	0.37

Abbreviations: EBNA = Epstein-Barr virus nuclear antigens; MS = multiple sclerosis; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II.
[a]EBNA1 for NHS/NHSII and Swedish MS Study; total EBNA for Tasmanian MS Study.

among ever smokers compared to those who never smoked (test for interaction, $p = 0.001$) (table 4). Similarly, among individuals with low anti-EBNA Ab titers (≤ median), there was no association between ever smoking and MS risk, while individuals with high anti-EBNA Ab titers (> median) had a significant 70% increased risk associated with ever smoking (table 4). Similar results were obtained when considering pack-years of smoking using the NHS/NHSII and Tasmanian data (p for interaction = 0.02). Tests of heterogeneity did not suggest between-study differences for the interaction or MS risk associated with anti-EBNA titers stratified by *HLA-DR15*.

The increased risk of MS associated with smoking did not differ by *HLA-DR15* (table 4). However, there was evidence of differences in effects between studies (p for heterogeneity <0.05). In the NHS/NHSII, the increased MS risk appeared to be limited to *HLA-DR15*-positive individuals, whereas, in the Tasmanian and Swedish studies, the increased MS risk was observed among *HLA-DR15*-negative individuals (table 4).

Finally, as previously reported in the NHS/NHSII,[5] the association between increasing anti-EBNA Ab titers and increased MS risk did not differ significantly by *HLA-DR15* status in any individual study or pooled analyses (table 4). However, there was again a suggestion of heterogeneity in effect estimates. A stronger effect of anti-EBNA titers was seen among *HLA-DR15*-negative individuals in the Tasmanian study, while in the NHS/NHSII and Swedish studies, a slight increase in the effect of anti-EBNA titers was seen in *HLA-DR15*-positive compared to *HLA-DR15* negative individuals.

Combined effects of EBNA titers and smoking. Examining the combined effects of smoking and anti-EBNA Ab titer suggested a super-multiplicative effect, never smokers with the highest anti-EBNA Ab titers had a fourfold increase in MS risk (RR = 4.1, 95% CI = 2.0–8.3, $p < 0.001$), while ever smokers with the highest anti-EBNA Ab titers had a sevenfold increased MS risk (RR = 7.4, 95% CI = 3.6–15.0, $p < 0.001$), compared to never smokers with the lowest anti-EBNA Ab titers (figure).

DISCUSSION

In this investigation, including data from 3 studies of risk factors for MS, we found that the well-established association between anti-EBNA Ab titers and MS risk varied based on smoking history. The association between increasing standard-

Table 3 Associations between anti-EBNA titers, *HLA-DR15*, smoking history at diagnosis,[a] and risk of MS

All participants	Nurses' Health Studies, OR (95% CI)	Tasmanian MS Study, OR (95% CI)	Swedish MS Study, OR (95% CI)[a]	Pooled, OR (95% CI)	*p* Value
Anti-EBNA Ab titer[b] (per standardized unit)	1.9 (1.4–2.6)	3.3 (2.3–4.8)	3.0 (1.8–5.0)	2.6 (1.8–3.8)[c]	<0.001
HLA-DR15 positive vs negative	2.9 (2.0–4.2)	3.6 (2.2–6.0)	3.8 (2.1–6.7)	3.2 (2.5–4.2)	<0.001
Smoking (ever vs never)	1.4 (1.0–2.0)	1.5 (1.0–2.4)	1.4 (0.8–2.4)	1.5 (1.1–1.9)	0.002

Abbreviations: CI = confidence interval; EBNA = Epstein-Barr virus nuclear antigens; MS = multiple sclerosis; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II; OR = odds ratio.
[a]In the Swedish MS Study, smoking history at time of diagnosis was estimated from smoking at time of blood draw as described in Methods.
[b]EBNA1 for NHS/NHSII and Swedish MS Study; total EBNA for Tasmanian MS Study.
[c]p for heterogeneity <0.05, random effects model estimate.

Table 4 Associations between anti-EBNA titers and risk of MS according to smoking history[a] and association between anti-EBNA titers or smoking history and MS according to *HLA-DR15* positivity

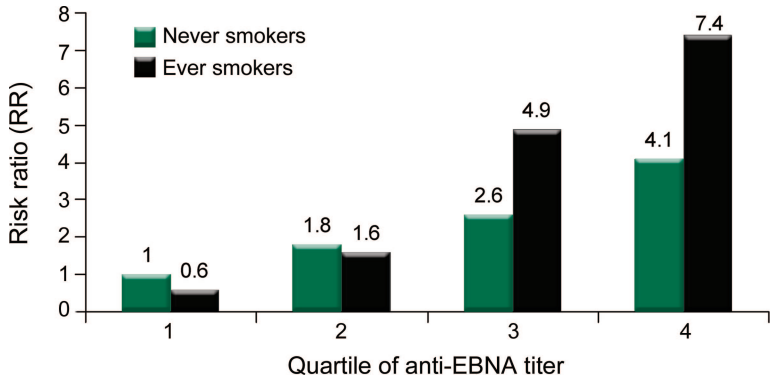
	Nurses' Health Study, OR (95% CI)	Tasmanian MS Study, OR (95% CI)	Swedish MS Study, OR (95% CI)	Pooled, OR (95% CI)	*p* Value
Estimates of OR of MS for increasing anti-EBNA Ab titer[b]					
Never smokers	1.4 (1.0–2.0)	2.6 (1.6–4.1)	2.0 (1.1–3.4)	1.8 (1.4–2.3)	<0.001
Ever smokers	3.0 (1.8–4.9)	4.9 (2.6–9.0)	7.6 (2.4–23.7)	3.9 (2.7–5.7)	<0.001
***p* Value for interaction**	0.02	0.1	0.04	0.001	
***HLA-DR15* negative**	1.5 (1.1–2.2)	4.1 (2.3–7.5)	2.3 (1.2–4.3)	2.4 (1.3–4.3)[c]	0.005
***HLA-DR15* positive**	2.1 (1.3–3.4)	1.9 (1.1–3.4)	3.3 (1.4–8.0)	2.2 (1.6–3.0)	<0.001
***p* Value for interaction**	0.31	0.07	0.49	0.95	
Estimates of OR of MS for smoking					
***HLA-DR15* negative**	1.0 (0.6–1.7)	1.8 (0.9–3.4)	2.4 (1.0–5.5)	1.4 (1.0–2.0)	0.07
***HLA-DR15* positive**	1.9 (1.2–3.2)	1.0 (0.5–2.1)	0.9 (0.4–2.1)	1.4 (1.0–2.0)	0.08
***p* Value for interaction**	0.07	0.26	0.1	0.67[c]	
Low anti-EBNA Ab titer	0.8 (0.5–1.4)	0.3 (0.09–1.2)	1.1 (0.5–2.4)	0.97 (0.7–1.3)	0.84
High anti-EBNA Ab titer	2.4 (1.1–5.2)	1.3 (0.8–2.4)	1.9 (0.8–4.8)	1.7 (1.1–2.6)	0.004
Estimates of OR of MS for *HLA-DR15* positivity					
Never smokers	2.0 (1.2–3.4)	4.9 (2.2–10.9)	6.7 (2.6–17.0)	3.8 (1.8–8.1)[c]	0.001
Ever smokers	3.8 (2.3–6.4)	2.7 (1.4–5.3)	2.6 (1.2–5.4)	3.2 (2.2–4.5)	<0.001
Low anti-EBNA Ab titer	1.6 (0.9–2.9)	4.9 (1.5–15.7)	3.8 (1.7–8.6)	2.4 (1.6–3.7)	<0.001
High anti-EBNA Ab titer	4.7 (2.2–10.3)	2.2 (1.2–4.1)	2.6 (1.0–6.3)	2.9 (1.9–4.4)	<0.001

Abbreviations: CI = confidence interval; EBNA = Epstein-Barr virus nuclear antigens; MS = multiple sclerosis; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II; OR = odds ratio.
[a]Smoking history at time of diagnosis. In the Swedish MS Study, smoking history at time of diagnosis was estimated from smoking at time of blood draw as described in Methods.
[b]EBNA1 for NHS/NHSII and Swedish MS Study; total EBNA for Tasmanian MS Study.
[c]*p* for heterogeneity <0.05, random effects model estimate.

ized anti-EBNA Ab titer and increased MS risk appeared to be approximately twofold greater among ever smokers compared to never smokers. Consistent with this, the 50% increased MS risk associated with ever smoking seemed to be limited to those with high anti-EBNA Ab titers. This effect was not dependent on *HLA-DR15* status; estimates for this interaction were not different between *HLA-DR15*-positive and *HLA-DR15*-negative individuals. Also, we did not observe modification by smoking of the association between *HLA-DR15* and MS risk, and, consistent with previous published work in the NHS,[5] no significant interaction between *HLA-DR15* and anti-EBNA Ab titer was observed. Unlike the consistent finding of an increased risk of MS associated with anti-EBNA Ab titers, particularly in those with a history of smoking, the effect of smoking and anti-EBNA titers stratified by *HLA-DR15* was not consistent between studies and seems mostly likely due to chance. Therefore, it is difficult to interpret the potential implications of such between-study differences. The finding of a significant interaction between anti-EBNA Ab titers and smoking is interesting in the context of results of 3 studies of risk factors for Hodgkin lymphoma, which is subtyped by the presence or absence of EBV. In these studies, risk of EBV-positive Hodgkin lymphoma was reported to be more strongly associated with smoking than EBV-negative Hodgkin lymphoma or total Hodgkin lymphoma,[15-17] also suggesting a possible biologic interaction between EBV and smoking.

We observed a pooled 50% increased risk of MS associated with smoking, consistent with a recent meta-analysis.[18] However, upon adjustment for anti-EBNA Ab titers, this association was no longer evident. This, in combination with the observation of a significant statistical interaction, may support the hypothesis of a common biologic pathway whereby some component of cigarette smoke modulates either EBV infection or the host immune system's response to EBV infection. Our data support a supermultiplicative effect suggesting that the association between high anti-EBNA immunoglobulin G Ab titers and MS risk is enhanced by smoking.

Figure **Risk of multiple sclerosis (MS) associated with increasing quartile of antibody titers to the Epstein-Barr virus nuclear antigens (anti-EBNA)* according to smoking status†**



†Smoking history at time of diagnosis. In the Swedish study, smoking history at time of diagnosis was estimated from smoking at time of blood draw as described in Methods. *EBNA1 for Nurses' Health Studies and Swedish study; total EBNA for Tasmanian study. Number of cases and controls included for never smokers: Q1 = 30/126, Q2 = 48/107, Q3 = 35/68, Q4 = 46/56; and ever smokers: Q1 = 17/100, Q2 = 51/126, Q3 = 65/76, Q4 = 85/63. Numbers may not sum to total number of cases and controls due to missing values.

Experimental or observational data addressing possible biologic mechanisms of interaction between EBV and smoking are sparse. The plausibility of this interaction may, however, be supported by the fact that commonalities exist related to consequences of exposure to nicotine or EBV. For example, EBV activation and nicotine metabolism have been shown to have shared molecular pathways including Jun-c-kinase,[19-21] MAPK,[22-24] PKC,[22,25,26] and NF-κB.[25,27,28] Additionally, changes in immune cell profiles may show some similar characteristics that are relevant for MS. Although $CD4^+$ T-cells are clearly implicated in the pathogenesis of MS, to a lesser extent, experimental and observational evidence also support a role for $CD8^+$ T-cells.[29-32] EBV infection elicits strong and persistent epitope-specific $CD8^+$ T-cell responses,[33] and it has been reported that smoking, particularly heavy smoking, may increase $CD8^+$ T-cell counts, although this finding has not been consistent.[34]

There are some limitations to these analyses. The measures of exposure assessment were somewhat different between the studies. These differences may have contributed to the heterogeneity of results across studies, but because all the analyses were stratified by study, they would not induce any spurious association, or any spurious interaction between the exposures of interest. A further cause of concern is that we could not account for changes over time in smoking behavior or anti-EBNA titers. Smoking behavior, however, tends to be stable in adults and data from the NHS cohorts suggest that there is little change in smoking behavior over this age range. Specifically, in the NHS cohorts, it appears that there is little change in smoking behavior as a result of MS diagnosis—only 1 woman reported initiating smoking and 1 quitting smoking in the time between diagnosis and blood draw. Similarly, in the Tasmanian study, only 1 individual who was not smoking at the time of diagnosis reported being a current smoker at the interview date. Finally, only a small proportion of individuals had anti-EBNA Ab titers measured before the disease onset. However, after primary EBV infection, anti-EBNA titers increase and become detectable within months and then remain elevated indefinitely[35] and the association between anti-EBNA titers and MS was similar in longitudinal and cross-sectional studies.[3] In our data, anti-EBNA titers remained stable relative to disease duration regardless of smoking history (data not shown). This suggests that assessing anti-EBNA titers after disease diagnosis is unlikely to explain our findings. We did not just adjust for race/ethnicity as these populations tended to be relatively homogenous. The NHS cohorts are the most diverse and still 95% of the cohort participants reported Caucasian ancestry. Because of the ethnic homogeneity, we cannot generalize these findings to other racial or ethnic groups. We also were not able to adjust for other potential confounders, such as vitamin D. However, it seems unlikely that confounding explains the finding of an interaction between smoking and anti-EBNA Ab titers. If such a factor existed, it would need to be associated with increased anti-EBNA Ab titers only among ever smokers. There is no evidence to suggest that vitamin D affects EBNA titers or that we would expect a different effect based on smoking history. Finally, the measured EBNA antibodies and time of smoking ascertainment (diagnosis vs blood draw) were different between studies. However, we conducted, as described, several analyses that suggest differences would have had minimal impact on our findings and the consistency across the studies for the main findings provides some empirical evidence against important effects of interstudy exposure ascertainment.

The consistency of the finding of a supermultiplicative interaction between anti-EBNA Ab titers and a history of smoking in 3 distinct, geographically diverse studies provides support that this finding is not due to chance. Further investigations are warranted to understand the potential mechanism underlying this finding.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Claire Simon.

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DISCLOSURE

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